EXPERIMENTAL STUDY OF POLYDISPERSIVE BACTERIAL AEROSOLS

REPORT 1

Theory of the Method for Determining the Survival of Microorganisms in a Polydispersive Bacterial Aerosol

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REPORT I

Theory of the Method for Determining the Survival of Microorganisms in a Polydispersive Bacterial Aerosol

[Following is the translation of an article by V. P. Zhalko-Titarenko, Kiev Institute of Microbiology and Epidemiology, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), #10, 1964, pages 61-66. It was submitted on 1 July 1963. Translation performed by Sp/7 Charles T. Ostertag Jr.]

The struggle with droplet infections is one of the most difficult and urgent problems of modern medical science. Therefore a study of the main link in the process of transmitting the causative agent -- the period of its residence in the air -- is drawing the attention of many native (Rechmenskiy, Vershigora, Vlodavets, Bolotovskiy and others) and foreign (Ferry et al., Harper et al., Rosebury et al., Wells, Webb and others) investigators.

The causative agents of droplet infections, upon entry into the air form a dispersed system -- a bacterial aerosol (or virus aerosol if the causative agent is a filterable virus). The bacterial aerosol is made up of a dispersion (suspension) stage -- the particles of the aerosol, and a dispersion medium -- the air. The make-up of the suspension stage, that is the particles, in bacterial systems is complex. If the aerosol was formed from a cellular suspension[1] then its particles will be made up of droplets of the dispersion medium of the suspension with the bacterial cells included in them. [1. In subsequent accounts the cellular suspension dispersed in the air will be called the initial suspension.] Thus the aerosol particle itself also represents a dispersed system with a liquid medium. This circumstance exerts an influence on the kinetic stability of the aerosol and on the fate of the microbes included in the particles.

As a rule, the dispersing of liquids leads to the formation of polydisperse systems, that is, aerosols containing particles of various size. And only under specific experimental conditions is it possible to obtain monodisperse systems in which all the particles are almost equal in size and contain the same number of cells. Aerosols produced by man

[illegible], sneezing, talking) are distinguished by a sharp degree of [illegible] -- the sizes of the particles fluctuate from several [illegible] large droplets and clots of mucus, the diameter of which [illegible] millimeters. With the help of such partici[illegible] that are various [illegible] infection of the aerial route takes place under natural con[illegible] In connection with this, there is interest in the study of poly[illegible] bacterial aerosols as a more complete model of the aerial[illegible] mechanism of transmitting infection.

[illegible]ever, the study of polydispersive bacterial aerosystems is made [illegible] due to undeveloped methods for determining the survival of the [illegible] ents in them. The relatively simple methods of determining [illegible] monodisperse systems (Ferry et al., Harper et al., Webb) [illegible] cannot be used in tests with polydisperse aerosols, since in [illegible] there is no calculation of the particle size and the number [illegible] contained in it. At the same time it is also impossible to [illegible] only to a study of survival in monodisperse systems, since the [illegible] of existence for microbes in particles of various size are not [illegible] Thus the development of methods for determining survival in a [illegible] aerosol is dictated by scientific necessity.

[illegible] series of works, beginning with this report, we have made an [illegible] resolve this problem on a model of an aerosol of the diphtherial [illegible] The present report contains the results of the theoretical [illegible] of the method and its basis.

[illegible]der to measure the survival of the causative agent, it is [illegible] know how many bacterial cells there are in all and how many [illegible] living. Having information of the concentration of live cells [illegible] (C_N) and its overall cellular concentration (C_c), it is [illegible] express, by the ratio of these values, the "specific weight" [illegible] in their overall mass:

$$\theta_0 = \frac{C_N}{C_0} \qquad (1)$$

[illegible] the degree of survival of the causative agent in an [illegible]

[illegible] termination of the concentration of live microbes (C_N) in a [illegible] aerosol presents a number of difficulties about which we will [illegible]. The study of this problem made it possible to select as the [illegible] for a soluble granular aerofilter made out of thin powder [illegible] te. By passing the aerosol through this powder it is

possible to achieve the deposition of almost all the particles on it. Then the powder was dissolved in a sterile isotopic solution, from which inoculations were made in dishes with a thick nutrient medium with a subsequent germination and computation of colonies for the purpose of calculating the value of C_N.

The overall concentration in a polydisperse aerosol may be expressed by the product of the concentration of particles (the so-called calculating concentration) -- C_c and the average number of microbes in a particle -- h :

$$C_0 = C_c \cdot h . \tag{1}$$

From here the formula (1) may be rewritten in the following manner:

$$\theta_0 = \frac{C_N}{C_c \cdot h} . \tag{2}$$

The calculating concentration C_c is easily determined in a VDK constant ultramicroscope (Deryagin and Vlasenko). A method for determining the average number of bacteria in a particle has hardly been developed, therefore we had to conduct several investigations in this area. A solution was found for the problem in a special method for staining the precipitate of the particles and getting it on a clean microscope slide. The preparation prepared in such a manner was subjected to microscope examination and a calculation was made of the number of bacterial cells in the particles.

On the whole, the degree of survival θ_0 was determined simultaneously by three methods: 1) the concentration of live microorganisms was determined by filtration of the aerosol through sodium alginate with a subsequent inoculation and calculation of colonies; 2) the calculating concentration of the aerosol was established in a VDK device; 3) the average number of microorganisms in the particle was measured by means of a direct count under a microscope in special preparations with the precipitate of the particles.

The proposed method is lacking in a determination of the limits of adaptability. One of the existing conditions limiting the use of the method may be the presence in the aerosol of fractions of particles that do not contain microbes. In this case "fractions of particles" implies all the particles of a specific size. With such a structure of aerodispersion, there could occur, for example, a final desiccation of the nonmicrobial droplets already after the first measurings in the aerosol and with the following measurements a very sharp reduction of the calculating concentration would be recorded. The index θ_0 would turn out higher than actual. Consequently it is necessary to have even if only a relative assurance that the aerosystem being investigated does not contain fractions of nonmicrobial particles. We are striving to set up conditions under which the nonmicrobial droplets will be organized.

All things considered, the spraying of a liquid represents a breaking up of it into smaller volumes -- particles. It is clear that in the breaking up of the suspension the cellular concentration in the particles will be the same as in the whole. However, this rule is preserved only up to a certain limit. In any suspension during separation into all smaller volumes it is possible to go up to that degree at which in the particle there remains only one cell (while preserving the same value of the cellular concentration). If such a particle is divided into two or a greater number of parts, the microbe will be in only one of these and the remaining ones will consist of the pure dispersing medium of the initial suspension. Thus, the minimum size of a particle, in which one cell remains while preserving the value of the concentration of the initial suspension, is critical. The cellular concentration of the suspension (K) is the ratio of the number of bacteria (M) to the volume of the suspension in which they are distributed (ϕ):

$$K = \frac{M}{\phi} . \qquad (3)$$

Upon achieving, as a result of the breaking down of the suspension, a value equal to the critical volume ϕ_{cr}, there turns out to be one cell in the particle:

$$K = \frac{1}{\phi_{cr}} ,$$

From where

$$\phi_{cr} = \frac{1}{K} . \qquad (4)$$

Since

$$\phi_{cr} = \frac{4}{3} \pi r_{cr}^3 ,$$

it is possible to write the formula (4) in the following manner:

$$\frac{4}{3} \pi r_{cr}^3 = \frac{1}{K}$$

from where

$$r_{cr} = \sqrt{\frac{3}{4\pi K}} \quad . \tag{5}$$

It is apparent that during spraying, fractions of nonmicrobial droplets will not be formed if they are all equal or exceed the critical size.

The elementary computations presented have significance only in case the diameter of the particle is known to be greater than the length of the microbe. Castleman and Zauter experimentally established that the minimum average diameter of droplets of water during its spraying comprises $10 \cdot 10^{-4}$ cm, that is, it considerably exceeds the length of almost all pathogenic bacteria. This value makes it possible to determine another critical condition for the adaptability of the method. Thus, in the formula (4), in place of ϕ_{cr} we place its numerical value, corresponding to a droplet with a diameter of $10 \cdot 10^{-4}$ cm:

$$\phi_{cr} = 522 \cdot 10^{-12}\ cm^3;\ K = \frac{1}{522 \cdot 10^{-12}} = 1.92 \cdot 10^9\ cells/cm^3.$$

The physical significance of these critical conditions consists of the following: If the concentration of the initial suspension equals or exceeds $1.92 \cdot 10^9$ cells/cm^3, then in general, fractions of nonmicrobial particles cannot be formed. However, the absence of nonmicrobial fractions may be guaranteed during smaller concentrations of the initial suspension if the minimum radius of the particles of the aerosol that are formed by this spraying satisfy the condition (5).

The specific limitation for determining the average number of cells in a particle (and consequently the entire method as a whole) may turn out to be that such a multitude of them in large particles does not submit to distinction under a microscope. Particles with 30-50 microbes are practicable for calculation. Usually such large particles, saturated with microbes, settle rapidly, and in 20-30 minutes are already difficult to find in an aerosol. In some cases this permits the broadening of the area of applicability of the method, if the initial value of the average number of microbes in the particle is determined by the method of extrapolation. The table presents the data from five determinations of the initial value of the average number of microbes in a particle in aerosols obtained from suspensions of a various concentration under similar conditions of spraying. The last column of the table contains the values for the coefficient of the ratio of the average number of microbes in the particle and the cellular

concentration of the initial suspension:

$$u = \frac{h}{K}. \qquad (6)$$

The coefficient u for all the determinations lies within the limits of $1.\ldots \cdot 10^{-9}$ -- $1.06 \cdot 10^{-9}$. This in general can be considered as sufficient foundation to view the relationship $\frac{h}{K}$ as a more or less constant value under constant conditions of spraying.

Thus, if there is assurance in the identity of the conditions of spraying, the initial value of h may determine, without turning to direct calculation, which of the values of particles or concentrations of microbes proves to be impossible. For this, tests are set up with smaller concentrations of the initial suspension and they determine the value of the coefficient u according to the formula (6). Knowing the value of u, it is not difficult to calculate by the same formula the initial value of h with the same concentration of the initial suspension, which is essential.

The general data presented on the method of determining the survival of causative agents in a polydisperse aerosol touch on the problem concerning the physical structure of a bacterial aerosol and are based on definite theoretical concepts concerning this structure. Contemporary knowledge on the construction of particles of a bacterial aerosol and the entire system as a whole remains very limited. This hampers not only the development of methods of investigation, but also an understanding of those processes taking place in the immediate vicinity of the bacterial cell and in the cell itself. The question, studied by Sonkin, concerning the proportionality of the size of the particles and the number of microbes in them served as our basis for the theoretical development of the conditions for the formation of nonmicrobial fractions in a bacterial aerosol. As already noted, the appearance of these fractions make it difficult to utilize the method proposed by us. However, such fractions may emerge not only in an experiment, but also in a natural situation during the transmission of infection. The conditions for the emergence of fractions of nonmicrobial particles are not changed by this. It is fully probable that the phenomenon mentioned actually influences the effectiveness of contamination, in some cases even making it impossible in spite of the presence of the causative agent and the formation of an aerosol.

It must be stipulated that the theoretical analysis of the conditions of the formation of nonmicrobial particles was performed without taking into consideration their Brownian movement and other factors causing a certain irregularity in the distribution of the microbes in the suspension. Therefore [illegible] presented grounds for the theory of the formation of n[illegible]

microbial particles bears the nature of a rough approximation. A subsequent analysis of this problem with a consideration of the theory of fluctuation makes it possible to more accurately find the conditions for the formation of nonmicrobial particles. In order to "cover" possible inaccuracies in the appraisal of the applicability of the suggested method for investigating polydisperse systems, we increased the concentration of the suspension being sprayed by 5-7 times in comparison with the critical suspension.

Conclusions

1. A formula has been proposed for calculating the survival of microbes in a polydisperse bacterial aerosol. It makes it possible to exclude the influence of the physical process in the aerosol on the survival index.

2. It has been established that the appearance of a considerable number of nonmicrobial particles in the aerosol can lead to the obtaining of a mistaken result.

3. It is shown theoretically that the upper limit of applicability of the method is an excessive number of microbes in the particle, making a quantitative calculation of them inaccessible, and the lower limit -- the critical radius of the system, depending on the concentration of the initial suspension, or the critical concentration of the initial suspension -- $1.92 \cdot 10^9$ cells/cm^3.

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Relationship of the cellular concentration of a sprayed suspension and the average number of bacteria in a particle.

No.	Cellular concentration of initial suspension	Average number of cells in particle	$\frac{h}{K}$
1	$2 \cdot 10^9$	2.98	$1.49 \cdot 10^{-9}$
2	$5 \cdot 10^9$	5.8	$1.16 \cdot 10^{-9}$
3	$7 \cdot 10^9$	9.2	$1.31 \cdot 10^{-9}$
4	$10 \cdot 10^9$	10.6	$1.06 \cdot 10^{-9}$
5	$14 \cdot 10^9$	16.2	$1.1[illegible] \cdot 10^{-9}$